provide the relationship between the distribution of amplitudes in focus and in xyt images (xyt-to-xyzt correction), thus unifying the three possible modes of spark sampling. The relative merits and shortcomings of the three modes will be discussed from this unified viewpoint.
Funded by NIAMS, NHLBI and NCRR (NIH).

3035-Pos Board B140
Isoproterenol Widens the Source of Release Flux Underlying Ca Sparks
Demetrio J. Santiago, Eduardo Rios, Thomas R. Shannon.
Our previous work [Biophys. J. 98(10):2111-20; Biophys. J. 98(3):102a] suggested that the diastolic ryanodine receptor (RyR) mediated leak (Jleak) from the sarcoplasmic reticulum (SR) of intact ventricular myocytes occurs in spark and non-spark forms. We further showed that the fraction of spark-mediated Jleak increases upon isoproterenol treatment in intact rabbit ventricular myocytes, suggesting that the effective sensitivity to cytosolic Ca is increased by RyR phosphorylation [Biophys. J. 98(3):102a]. We now present an extension of this work, focused on aspects of individual sparks taken from cells at matched [Ca] and SR load. Events were wider in isoproterenol (8.5% greater FWHM of the F/F0 profile) but had similar amplitudes than control. A backward reconstruction of the release flux density, when applied to average sparks assumed to be spherically symmetric, rendered a source that was wider for the isoproterenol event, indicating the recruitment of peripheral RyRs. A forward release flux reconstruction which recapitulates the steps of spark formation could not simultaneously fit the amplitudes and sizes of any of the two average sparks when using realistic radii for the junctional SR. This result may be interpreted as implying the existence of RyRs, peripheral to and perhaps outside the couplon. Compounded with the increased CICR sensitivity upon isoproterenol treatment (see above), the greater spark width of isoproterenol events may increase the probability of Ca wave generation.

3036-Pos Board B141
Activation of Calcium Sparks in Resting Cardiomyocytes by β-Adrenergic Stimulation May Involve CaMKII and nNOS
Daniel Gutierrez, Jakob Ogrodnik, Ernst Niggli.
It has been reported that during β-adrenergic stimulation of cardiac myocytes, phosphorylation of Ca^{2+} release channels (ryanodine receptors, RyRs) by PKA and/or CaMKII may result in arrhythmogenic diastolic Ca^{2+} leak (as elementary Ca^{2+} release events, Ca^{2+} sparks) from intracellular Ca^{2+} stores (the sarcoplasmic reticulum, SR). Using confocal Ca^{2+} imaging, we have recently shown that β-adrenergic stimulation by 1 μM isoproterenol (ISO) increases the Ca^{2+} spark frequency several-fold in quiescent, whole-cell voltage-clamped guinea-pig myocytes, without altering SR Ca^{2+} content. As this occurs without variations of the diastolic intracellular Ca^{2+} concentration, this observation suggests a sensitization of the RyRs. Experiments with protein kinase inhibitors (KN-93 and H89) indicated an involvement of CaMKII in the change of spark frequency. Surprisingly, but in line with the kinase inhibitor experiments, increasing cAMP production and PKA activity by direct stimulation of adenylate cyclase with forskolin (1 μM) did not significantly elevate Ca^{2+} spark frequencies under the same experimental conditions. Further experiments revealed that the change in sensitivity of the RyRs upon β-adrenergic stimulation may be linked to nitric oxide (NO), as pre-incubation of the cells with the NOS inhibitor L-NAME (500 μM) prevented the increase of the Ca^{2+} spark frequency without dramatic changes of SR Ca^{2+} content. Using the nNOS specific inhibitor AAAN (100 μM) resulted in analogous observations, suggesting that the nNOS isoform, located in close proximity of the RyRs, may be involved in this signaling pathway. Taken together, the results suggest the presence of a non-classical pathway linking β-adrenergic stimulation of cardiac myocytes to enhanced activity of the RyRs. Preliminary pharmacological evidence indicates that the pathway includes both, CaMKII and nNOS as important components. Supported by SNF.

3037-Pos Board B142
B-Adrenergic Stimulation Accelerates Local Recovery of Cardiac Ca^{2+} Release
Ona Liu, Hena Ramay, Eric A. Sobie.
In cardiac myocytes, Ca^{2+} sparks terminate reliably and exhibit time-dependent refractoriness after termination. Compelling evidence suggests that dynamic local changes in SR [Ca^{2+}] play an important role in the control of these processes. We examined Ca^{2+} spark refractoriness by exposing fluo-3 loaded quiescent rat ventricular myocytes to 50 nM ryanodine, recording Ca^{2+} sparks with a confocal microscope, and analyzing the repeated sparks that were produced at a limited number of ryanodine receptor (RyR) clusters. Previous experiments showed that altering RyR sensitivity (caffeine or tetracaine) influenced the time between consecutive sparks but did not affect the recovery of spark amplitude (time constant = ~100 ms in all cases). Here we examined repeated Ca^{2+} sparks after application of 100 nM isoproterenol to determine how β-adrenergic stimulation influences spark restitution. Isoproterenol dramatically decreased the median interval between consecutive sparks (192 ms vs. 280 ms in control) and led to faster recovery of Ca^{2+} spark amplitude (time constant = 58 ms). Mechanisms underlying these results were explored through simulations with an established mathematical model of the Ca^{2+} spark. Simulations showed that faster SR refilling led to earlier triggering of Ca^{2+} sparks due to the greater flux of Ca^{2+} through each open RyR, but this effect was insufficient to explain the experimental data. The results could be reproduced if we assumed that isoproterenol both increased the rate of local SR refilling and increased RyR sensitivity. Together, our results indicate that β-adrenergic stimulation influences both: 1) Ca^{2+} spark amplitude recovery, through changes in the time course of local SR refilling; and 2) Ca^{2+} spark triggering, through changes in both refilling and RyR sensitivity.

3038-Pos Board B143
Beta-Adrenergic Stimulation Increases the Intra-Sarcoplasmic Reticulum Ca Threshold for Spontaneous Ca Waves
Timothy L. Domeier, Joshua T. Maxwell, Lothar A. Blatter.
Beta-adrenergic signaling induces positive inotropic effects on the heart that frequently associate with spontaneous arrhythmogenic Ca release events including Ca waves. It remains unclear if the greater incidence of Ca waves is due to increased sarcoplasmic reticulum (SR) Ca content ([Ca]SR) or a change in the function of ryanodine receptors. To address this controversy we utilized dynamic [Ca]SR measurements (fluo-5N) to test if beta-adrenergic stimulation alters the [Ca]SR level where Ca waves initiate (wave threshold) during rest after action potential stimulation. Under control conditions [Ca]SR was progressively increased to the wave threshold via incremental increases in pacing frequency in a high extracellular Ca (7 mM) environment. In the presence of the beta-adrenergic agonist isoproterenol (ISO, 1 microM) [Ca]SR increased and Ca waves were observed. When [Ca]SR was subsequently lowered using low extracellular Ca (1 mM) and SERCA inhibition (3 microM cyclopiazonic acid), Ca waves were no longer observed, even at [Ca]SR levels above the control wave threshold. In parallel experiments we found that resting cytosolic [Ca] (indo-1) was similar between the respective experimental conditions. Indirect assessment of [Ca]SR using the amplitude of the cytosolic Ca transient induced by 10 mM caffeine confirmed our observation that in the presence of ISO Ca waves only occur when [Ca]SR is above the control wave threshold. Furthermore, spontaneous Ca spark measurements (fluo-4) showed a tendency towards spark inhibition in the presence of ISO at experimentally matched [Ca] SR. Together, these data show that acute beta-adrenergic stimulation increases the [Ca]SR threshold for Ca waves, and therefore the primary cause of Ca waves is the robust increase in [Ca]SR above this higher threshold level. Elevation of the [Ca]SR wave threshold may be interpreted as a protective mechanism against pro-arrhythmogenic Ca release during beta-adrenergic stimulation.

3039-Pos Board B144
β-Adrenergic Receptor Stimulation of ROS Production Generates Spontaneous Ca^{2+} Waves in Rabbit Ventricular Myocytes
Elisa Bovo, Stefan R. Mazurek, Stephen L. Lipsius, **Aleksey V. Zima**.
Stimulation of β-adrenergic receptors (β-AR) leads to positive inotropic effects, but also can generate pro-arrhythmogenic spontaneous Ca^{2+} waves. We investigated the role of reactive oxygen species (ROS) production in the generation of Ca^{2+} waves during β-AR stimulation in rabbit ventricular myocytes. In electrically stimulated myocytes, isoproterenol (ISO; 0.1 μM) increased Ca^{2+} transient amplitude during systole, sarcoplasmic reticulum (SR) Ca^{2+} load and the occurrence of spontaneous Ca^{2+} waves during diastole. These effects, however, developed at different time points during ISO application. While SR Ca^{2+} release and load reached maximum after 3 min, Ca^{2+} waves did not appear until 6-12 min after ISO application. Measurements of intra-SR free Ca^{2+} ($[Ca^{2+}]_{SR}$) with Fluo-5N showed an initial increase of SR Ca^{2+} load from 0.9 to 2.1 mM followed by a gradual decline to 1.4 mM after 12 min of ISO application. This decline of $[Ca^{2+}]_{SR}$ was not due to decreased SERCA activity, but instead was the result of increased SR Ca^{2+} leak in the form of Ca^{2+} waves. SR Ca^{2+} leak, measured as a decline of $[Ca^{2+}]_{SR}$ after SERCA inhibition, was increased by 30% after 6-12 min of ISO application. Moreover, ISO significantly increased ROS production. ROS scavenger Tiron and superoxide dismutase mimetic MnTBPA abolished the ISO-mediated ROS production. Tiron (10 mM) or MnTBPA (20 μM) significantly decreased the occurrence of Ca^{2+} waves during ISO application and partially prevented ISO-mediated SR Ca^{2+} leak, but did not affect ISO-mediated increase in SR Ca^{2+} load or Ca^{2+} transient amplitude. ROS donor t-butyl peroxide (100 μM) elicited Ca^{2+} waves that were dependent on elevated SR Ca^{2+} load. These results demonstrate that β-AR-mediated ROS production acts in